Chemistry of Natural Compounds, Vol. 45, No. 2, 2009

A BICYCLO[3.2.1]OCTANOID NEOLIGNAN AND TOXICITY OF THE ETHANOL EXTRACT FROM THE FRUIT OF *Ocotea heterochroma*

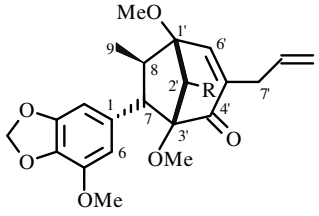
Luis E. Cuca*, Paula Leon, and Ericsson D. Coy

UDC 547.972

A new bicyclo[3.2.1]octanoid neolignan rel-*(7*S*,8*R*,1′*S*,2′*R*,3′*S*)-Δ^{8}′-2′-hydroxy-5,1′,3′-trimethoxy-3,4-methylenedioxy-7,3′,8,1′-neolignan (**1**) was isolated from ethanol extract from the fruit of* Ocotea heterochroma *Mez & Sodiro ex Mez as well as the known compounds* β*-friedelanol (**2**),* meso*-dehydroguaiaretic acid (**3**), and yangambin (**4**), whose structures were elucidated on the basis of their comprehensive spectroscopic analysis including 2D NMR data. Lethality bioassay using brine shrimp (*Artemia salina *Leach) was evaluated with the ethanol extract from the* Ocotea heterochroma*'s fruit. The toxicity of this extract was greater than the toxicity of those fractions obtained in a first solvent partition (benzene, ethyl acetate, and butanol subfractions) and that of a mixture of acetylated 2′-epimers from the new neolignan **1**.*

Key words: *Ocotea heterochroma*, Lauraceae, bicyclo[3.2.1]octanoid neolignan, lethality bioassay, *Artemia salina*.

Previous studies of secondary metabolites isolated from the *Ocotea* genus (Lauraceae) have revealed that this genus contains a wide range of neolignan-types: 2,5-diaryl-3,4-dimethyltetrahydrofuran, 3a-allyl-5-methoxy-3-methyl-2-veratryl-2,3,3a,4,5,6-hexahydro-6-oxobenzofuran, ketone, and bicyclo[3.2.1],1,2-diaryl and 1-aryl-2-cyclohexyl-propanoid among others [1–7]. The present work describes the extraction, purification, and structural determination of a new bicyclo[3.2.1]octanoid neolignan *rel*-(7*S*,8*R*,1′*S*,2′*R*,3′*S*)-Δ^{8}′-2′-hydroxy-5,1′,3′-trimethoxy-3,4-methylenedioxy-7,3′,8,1′-neolignan (**1**), as well as the known compounds β-friedelanol (**2**), *meso*-dehydroguaiaretic acid (**3**), and yangambin (**4**). Their structures were determined by extensive spectroscopic analysis. The toxicity of ethanol extract from the fruit of *O. heterochroma*, the benzene, ethyl acetate, and butanol subfractions, and a mixture of acetylated 2′-epimers from the neolignan **1** were evaluated by means of the lethality bioassay on brine shrimp (*Artemia salina* LEACH) by LC_{50} determination.



1, 5

1: R = OH
5: R = OAc

Compound **1** was obtained as colorless needles. Their EIMS spectrum showed a [M^{+}] at *m/z* 402. Its structure was defined by NMR analysis (Table 1), as well as by preparation of its acetylated derivative **5** as *rel*-(7*S*,8*R*,1′*S*,2′*R*,3′*S*)-Δ^{8}′-2′-hydroxy-5,1′,3′-trimethoxy-3,4-methylenedioxy-7,3′,8,1′-neolignan (**1**), isolated from the crude ethanol extract from *Ocotea heterochroma* Mez & Sodiro ex Mez. Its relative configuration was determined by NOE experiment.

Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Quimica, Laboratorio de Investigacion en Productos Naturales Vegetales, AA 14490, Cra 30 45-03, Ciudad Universitaria, Bogota D.C., Colombia, e-mail: lecucas@unal.edu.co. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 158–160, March–April, 2009. Original article submitted July 2, 2007.

0009-3130/09/4502-0179

TABLE 1. Chemical Shifts for Compound **1**[a]

C atom	δ_C[b]	δ_H[b] (J/Hz)	HMBC (^{13}C–^{1}H)	NOE[c]	NOE (^{1}H–^{1}H)
1	131.2			H-6′	N
2	110.2	6.50 (1H, s)		H-8	H-6S, H-2S, H-9S, H-7W
3	148.6		5-OMe, H-6, H-2		
4	131.5		H-7, H-8, H-6, H-2	5-OMe	N
5	142.9		H-7, H-6	H-2′	N
6	110.6	6.68 (1H, s)	H-7, H-6		
7	59.5	3.12 (1H, d, J = 8.5)	H-8, H-2′, H-9, H-7, H-6, H-2		
8	48.6	2.20 (1H, q, J = 7.3)	H-9, H-7, H-2′		
CH_3-9	17.2	1.16 (3H, d, J = 6.8)	H-9, H-7, H-8		
1′	86.0		H-9, H-8, 3′-OMe, H-2′, H-6′		
2′	75.5	4.05 (1H, s)	H-7, H-2′		
3′	94.4		H-7, H-1′		
4′-C=O	191.4				
5′	140.5				
6′	125.4	6.55 (1H, s)	H-8, H-7′, H-2′		
7′	34.5	2.75 (2H, ddd, J = 6.8, 9.4, 16.2)	H-9′, H-8′, H-6′		
8′	134.2	5.82–5.88 (1H, m)	H-7′, H-9′		
9′	117.7	5.10–5.25 (2H, m)	H-7′		
OCH_2O	101.2	5.88 (2H, s)	H-2		
3′-OMe	53.9	3.52 (3H, s)			
1′-OMe	54.5	3.33 (3H, s)			
5-OMe	56.8	3.82 (3H, s)			
OH		2.12 (1H, s)			

[a]C–H conectivities were ascertained by HMQC and neighboring H–H couplings by ^{1}H–^{1}H-COSY; [b]^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$); [c]irradiated proton; H-H interactions were shown with selective NOE (600 MHz).

The benzylic H-7 proton was found at δ_H 3.12 in the high field in compound **1**, which is due to placing the proton with respect to the carbonyl group in position 4′, since this group was *endo* to the carbonyl group where its anisotropic protection against H-7 is not exercised. The NOE experiment on proton H-8 δ_H 2.29 gave important evidence of the proposed configuration, since this showed that the proton H-8 was on the same plane (on presenting 3 signals – H-9, H-2 and H-6 – and one weak signal – H-7) of H-6 and H-2 protons, even though it did not couple directly to them (all of them were *endo* to the main bridge). The H-9 proton signal also appeared by coupling to H-8 directly, without needing (due to its spatial proximity) to be on the same plane for the intensification of its signal to take place (H-8 was *endo* to the main bridge and H-9 was *exo* to the main bridge). The H-7 weak signal showed that even though this proton coupled to H-8, it was found to be spatially far from the plane (H-7 was *exo* to the main bridge) promoting a weak H-7 signal. The relative configuration displayed by compound **1** explains the CH_3-9 group position, which was found in the high δ_H 1.16 ppm since it was *exo* to the main bridge and, as methyl was on the same plane as the bicycle's main OH group bridge, it was found to be influenced by this group′s inductive effect.

Lethality bioassay using brine shrimp (*Artemia salina* Leach). The ethanol extract from the fruit of *Ocotea heterochroma* showed that it had significant toxicity (bearing in mind that it is an extract) on comparing its LC_{50} (crude ethanol extract 28.1 ppm) value with those values obtained for the other fractions assayed, which came from the partition of the first extract (benzene fraction – 51.8 ppm and butanol fraction – 74.1 ppm), and for the mixture of the 2′-acetylated compounds from the new neolignan (ethyl acetate fraction – 45.4 and a mixture of 2′-epimers – 120 ppm). However, even though the toxicity presented by this extract is greater than that of its different fractions obtained by partition, these fractions′ toxicity values are also high, indicating that there is great potential in the bioactivity of this species in particular.

EXPERIMENTAL

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature in $CDCl_3$ on a Bruker Avance instrument operating at 600 and 150 MHz, respectively. EIMS were measured on a GC Hewlett Packard 6890

model coupled with selective mass detector 5973 at 70 eV. Melting points were recorded on a Fisher monoscope and are uncorrected. Si gel chromatography was made using silica gel 60 (0.2–0.5 mm Merck) for analytical work. Preparative TLC was carried out by using Merck silica gel 60 GF 254, Sephadex LH-20, and chromatotron 7924T Harrison Research model.

Plant Material. Fruits from *Ocotea heterochroma* were collected in the Colombian Cota County, Cundinamarca Department, Colombia. This plant is known locally as "yellow laurel." A voucher specimen is kept in the Colombian National Herbarium, at the Universidad National's Institute of Natural Sciences (Collection number: COL333382).

Extraction and Isolation. 1.7 kg dry *Ocotea heterochroma* fruit was macerated; 278.1 g ethanol extract was obtained by extraction at room temperature with 95% EtOH.

241.1 g ethanol extract from the *Ocotea heterochroma* fruit was then submitted to the partition process with 6 solvents (with increasing polarity) affording 6 extracts: petroleum ether (23.9%), benzene (15.2%), chloroform (2.8%), ethyl acetate (8.1%), butanol (15.3%), and water (13.5%).

The chloroform fraction was purified by CC with silica gel and eluted with a benzene:ethyl acetate mixture (8:2) to obtain a compound (264 mg): (7*S*′8*R*,1′*S*,2′*R*,3′*S*)-$\Delta^{8'}$-2′-hydroxy-5,1′,3′-trimethoxy-3,4-methylenedioxy-7,3′,8,1′-neolignan (**1**), colorless needles with 184–185°C melting point. 30 mg of **1** was submitted to acetylating with 0.5 mL pyridine and 1.5 mL acetic anhydride for 24 h to give 18 mg compound **2**.

***rel*-(7*S*,8*R*,1′*S*,2′*R*,3′*S*)-$\Delta^{8'}$-2′Hydroxy-5,1′,3′-trimethoxy-3,4-methylenedioxy-7,3′,8,1′-neolignan (1)**. Colorless needles from acetone, mp 184–185°C; UV (MeOH, λ_{max}, log ε): 213 (4.72), 243 (4.27), 283 (3.51); IR (KBr, ν_{max}, cm^{-1}): 3428, 3083, 2951, 2842, 1695, 1633, 1513, 1352, 1334, 1201, 1137, 1115, 1092, 1036, 869, 825, 750; EIMS *m/z* (%): 402(72) [M^+], 384 (2), 370 (3), 210 (17), 194 (100), 192 (37), 169 (66), 165 (26), 150 (11), 135 (9), 41 (3); ^{1}H NMR (600 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) see Table 1.

***rel*-(7*S*,8*R*,1′*S*,2′*R*,3′*S*)-$\Delta^{8'}$-2′-Acetoxy-5,1′,3′-trimethoxy-3,4-methylenedioxy-7,3′,8,1′-neolignan (5)**. IR (film, ν_{max}, cm^{-1}): 2937, 2840, 1749, 1694, 1635, 1512, 1453, 1435, 1374, 1322, 1223, 1138, 1095, 1065, 1003, 930, 824, 800, 733, 678; EIMS *m/z* (%): 444 (59) [M^+], 402 (2), 385 (5), 252 (2), 210 (100), 192 (57), 165 (16), 150 (5), 43 (14); ^{1}H NMR (600 MHz, $CDCl_3$, J/Hz): δ_H 6.43 (1H, s, H-2), 6.66 (1H, s, H-6), 3.20 (1H, d, J = 8.71, H-7), 2.29 (1H, q, J = 7.24, H-8), 1.22 (3H, d, J = 6.8, H-9), 5.25 (1H, s, H-2′), 6.58 (1H, s, H-6′), 2.2–2.5 (2H, m, H-7′), 5.70–5.85 (1H, m, H-8′), 5.20–5.36 (2H, m, H-9′), 5.94 (2H, dd, J = 7.37, 1.00, OCH_2O-3,4), 3.47 (3H, s, 3′-OMe), 3.25 (3H, s, 1′-OMe), 3.87 (3H, s, 5-OMe), 2.30 (3H, s, AcO).

Lethality Bioassay. The procedure was followed according to the reported method by McLaughlin et al. [8] for the lethality bioassay with brine shrimp (*Artemia salina* Leach). The ethanol extract from the fruit of *Ocotea heterochroma* and those of benzene, ethyl acetate, and butanol subfractions were submitted to lethality bioassay, using concentrations in 20, 40, 60, 80, and 100 ppm MeOH solutions of extracts. The acute toxicity of a 2′-acetylated epimer mixture from the new neolignan was also evaluated in 100, 200, 300, 400, and 500 ppm concentrations.

ACKNOWLEDGMENT

We thank the Chemistry Department of Universidad Nacional de Colombia and Plant Natural Product Laboratory for financing this research and the Fundacion Instituto de Inmunologia de Colombia (FIDIC), Bogota, for taking the spectra records.

REFERENCES

1. H. Lopez, A. Valera, and J. Trujillo, *J. Nat. Prod.,* **58**, 782 (1995).
2. A. Lacava and M. Yoshida, *Phytochemistry*, **46**, 741 (1997).
3. J. Aiba, O. R. Gottlieb, M. Yoshida, J. Mourao, and E. Gottlieb, *Phytochemistry*, **15**, 1031 (1975).
4. Q. Jesus-Morais, F. Assis, B. Cordeiro, M. Filho, W. Lima, L. Silva, T. Bozza, and C. Neto, *Planta Med.,* **66**, 211 (2000)
5. P. Romoff, M. Yoshida, and O. Gottlieb, *Phytochemistry*, **23**, 2101 (1984).
6. G. Carvalho, M. Yoshida, O. Gottlieb, and E. Gottlieb, *Phytochemistry*, **27**, 2319 (1988).
7. M. David, M. Yoshida, and O. Gottlieb, *Phytochemistry*, **36**, 491 (1994).
8. J. McLaughlin, M. Rogers, and J. E. Anderson, *Drug Inform. J.,* **32**, 513 (1998).